

Application No. 10/863,722
Response Date: December 18, 2006
Reply to Office Action: June 21, 2008

2

Amendments to the Specification:

On page 1 of this application, immediately following the title, please amend with the following paragraph:

REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Patent Application no. 09/213,770 filed December 17, 1998.

Please replace the paragraph beginning at page 5, line 8, with the following rewritten paragraph:

Influenza A viruses are classified into sub-types on the basis of two surface antigens, hemagglutinin (H) and neuraminidase (N). Three subtypes of the hemagglutinin (H1, H2, H3) and two sub-types of neuraminidase (N1, N2) are recognized among influenza A viruses that have caused widespread human diseases. Immunity to these antigens[[.]] reduces the likelihood of infections and lessens the severity of the disease if infection occurs.

Please replace the paragraph beginning at page 9, line 6, with the following rewritten paragraph:

The composition and manner of preparation of the mixture of RSV protein is fully described in US Patent Application No. ~~08/679,060, filed July 12, 1996, No. 6,020,182~~ and in published PCT Application WO 98/02457, the disclosures of which are incorporated herein by reference. As described therein, the mixture of RSV protein may be obtained by coisolating and copurifying the mixture from the virus. RSV cells are grown in a culture medium and separated from the culture medium. The F, G and M proteins are solubilized from the separated virus and the solubilized RSV protein are coisolated and copurified. Such coisolation and copurification may be effected by loading the solubilized protein onto an ion-exchange matrix, preferably a calcium phosphate matrix, specifically a hydroxyapatite matrix, and selectively relating the F, G and M protein from the ion-exchange matrix. The grown virus may first be

Application No. 10/663,722
Response Date: December 18, 2006
Reply to Office Action: June 21, 2006

3

worked with urea to remove contaminants without substantially removing F, G and M protein.

Please replace the paragraph beginning at page 10, line 31, with the following rewritten paragraph:

In each of the Figures, a common legend is used for identification of with immunogens used in the experiments for which the data is presented in the Figures as follows:

(a) phosphate buffered saline (PBS); (b) 200 µg PCPP adjuvant; (c) 1.5×10^5 pfu live RSV; (d) 200 to 400 HA units live influenza; (e) 5 µg ~~Fluzone~~ FLUZONE® vaccine (a split-antigen influenza vaccine) with PCPP adjuvant; (f) 1 µg RSV vaccine with PCPP adjuvant; (g) 5 µg ~~Fluzone~~ FLUZONE® vaccine plus 1 µg RSV vaccine with PCPP adjuvant; (h) 5 µg ~~Fluzone~~ FLUZONE® vaccine plus 1 µg RSV vaccine with no adjuvant; (i) 5 µg ~~Fluzone~~ FLUZONE® vaccine with no adjuvant; (j) 1 µg RSV vaccine with no adjuvant.

Please replace the paragraph beginning at page 11, line 34, with the following rewritten paragraph:

The mixture of F, G and M proteins of RSV used herein may be coisolated and copurified from RS virus. As described in the ~~aforesaid~~ USAN 08/679,060 U.S. Patent No. 6,020,282 and WO 98/02457, the virus is grown on a vaccine quality cell line, such as VERO cells and human diploid cells, such as MRC5 and WI38, and the grown virus is harvested. The fermentation may be effected in the presence of fetal bovine serum (FBS) and trypsin.

Please replace the paragraph beginning at page 13, line 12, with the following rewritten paragraph:

The influenza virus vaccine utilized herein is a sterile suspension prepared from influenza virus propagated in chicken embryos. The virus containing allantoic fluids, are harvested and inactivated with formaldehyde. The virus is then

Application No. 10/663,722
Response Date: December 18, 2006
Reply to Office Action: June 21, 2006

4

concentrated and purified in a linear sucrose density gradient solution, using a continuous flow centrifuge. The virus is then chemically disrupted using Glyco p-, Isooctylphenyl Ether (~~Triton~~ TRITON® X-100) producing a split-antigen. The split-antigen is then further purified by chemical means and suspended in sodium phosphate-buffered isotonic sodium chloride solution. Gelatin (0.05%) is then added as a stabilizer and thimerosol (1:10,000) is added as a preservative.

Please replace the paragraph beginning at page 13, line 26, with the following rewritten paragraph:

The commercial vaccine (~~Fluzone~~ FLUZONE®) as used herein was obtained from Connaught Laboratories, Swiftwater, PA.

Please replace the paragraph beginning at page 19, line 30, with the following rewritten paragraph:

A solution of 50% polyethylene glycol-8000 was added to an aliquot of virus concentrate prepared as described in Example 1 to give a final concentration of 6%. After stirring at room temperature for one hour, the mixture was centrifuged at 15,000 RPM for 30 min in a Sorvall SS-34 rotor at 4°C. The viral pellet was suspended in 1 mM sodium phosphate, pH 6.8, 2 M urea, 0.15 M NaCl, stirred for 1 hour at room temperature, and then recentrifuged at 15,000 RPM for 30 min. in a Sorvall SS-34 rotor at 4°C. The viral pellet was then suspended in 1 mM sodium phosphate, pH 6.8, 50 mM NaCl, 1% ~~Triton~~ TRITON® X-100 and stirred for 30 minutes at room temperature. The insoluble virus core was removed by centrifugation at 15,000 RPM for 30 min. in a Sorval SS-34 rotor at 4°C. The soluble protein supernatant was applied to a column of ceramic hydroxyapatite (type II, Bio-Rad Laboratories) and the column was then washed with five column volumes of 1 mM sodium phosphate, pH 6.8, 50 mM NaCl, 0.02% ~~Triton~~ TRITON® X-100. The RSV subunit composition from RSV subtype A, containing the F, G and M proteins, was obtained by eluting the column with 10 column volumes of 1 mM sodium phosphate, pH 6.8, 400 mM NaCl, 0.02% ~~Triton~~ TRITON® X-100.

Application No. 10/663,722
Response Date: December 18, 2006
Reply to Office Action: June 21, 2006

5

Please replace the paragraph beginning at page 20, line 20, with the following rewritten paragraph:

The influenza virus vaccine utilized herein is a sterile suspension prepared from influenza virus propagated in chicken embryos. The virus containing allantoic fluids, are harvested and inactivated with formaldehyde. The virus is then concentrated and purified in a linear sucrose density gradient solution, using a continuous flow centrifuge. The virus is then chemically disrupted using Glyco p-, Isooctylphenyl Ether (~~Triton~~ TRITON® X-100) producing a split-antigen. The split-antigen is then further purified by chemical means and suspended in sodium phosphate-buffered isotonic sodium chloride solution. Gelatin (0.05%) is then added as a stabilizer and thimerosal (1:10,000) is added as a preservative.

Please replace the paragraph beginning at page 20, line 34, with the following rewritten paragraph:

The commercial vaccine (~~Fluzone~~ FLUZONE®) as used herein was obtained from Connaught Laboratories, Swiftwater, PA.

Please replace the paragraph beginning at page 21, line 4, with the following rewritten paragraph:

Mice were bleed one day prior to the first immunization and also on days 22 and 28 of the study. Immunizations were done on days 1 and 22. Both immunizations were administered intramuscularly in the thigh muscle. Each immunization was done at two injection sites (both right and left thigh muscles; 0.05 ml/site). The dose of RSV vaccine was 1 µg total protein and the dose of ~~Fluzone~~ FLUZONE® vaccine was 5 µg total protein per dose. The RSV or ~~Fluzone~~ FLUZONE® vaccines were administered in the presence or absence of adjuvant. The adjuvant used was poly-di(carboxylatophenoxy)-phosphazene (PCPP) given at 200 µg/dose. Mice that received live RSV (A2 strain) as the immunogen were given 1.5 x 10⁶ pfu/dose intranasally. Mice that received live influenza virus (A/Taiwan Strain) as the immunogen were given 200 to 400 HAU/dose intraperitoneally. Virus challenge with

Application No. 10/663,722
Response Date: December 18, 2006
Reply to Office Action: June 21, 2006

6

either RSV or influenza was administered intranasally on day 29 using the same dose as given for the live virus immunized mice. All animals were sacrificed on day 33. Lungs were removed and frozen immediately in liquid nitrogen for later determination of virus titre.